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Cardiomyocyte specific CD38 deletion protects heart from acute myocardial infarction by activating Sirt3 signaling pathway

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Acute myocardial infarction (AMI) is serious disease with high morbidity and mortality worldwide. CD38 is an important metabolic enzyme and plays an important role in a variety of diseases. Our previous studies demonstrated that CD38 deficiency significantly reduced Ang-II-induced ventricular hypertrophy and cardiac ischemia-reperfusion injury. However, the roles of cardiomyocytic CD38 in acute myocardial infarction (AMI) remain unknown. Here, we reported that cardiomyocyte-specific CD38 deficiency ($CD38^{CKO}$) significantly improved heart functions in AMI. We observed that $CD38^{CKO}$ remarkably reduced the fibrosis at the peri-infarct area, and inhibited the apoptosis of cardiomyocytes in infarcted area by elevating the ratio of mitochondrial Bcl2/Bax expression and increased the expressions of the mitochondrial fusion proteins Mfn1 and Mfn2 in the early stage of AMI. Consistently, knockdown of CD38 protected hypoxia-induced apoptosis in cardiomyocytes by increasing the ratio of Bcl2/Bax expression and decreasing cleaved caspase-3. More importantly, 3-TYP, a Sirt3 inhibitor, significantly increased hypoxia-induced apoptosis in CD38-deficient primary cardiomyocytes. In conclusion, our results demonstrated that $CD38^{CKO}$ suppressed apoptosis of cardiomyocytes in the infracted area of heart via activating NAD^+/Sirt3-mediated signaling pathways.

Keywords CD38, Sirt3, Cardiomyocytes, Mitochondria, Apoptosis

Although the survival rate after acute myocardial infarction (AMI) has been significantly improved over the past few decades with the increase of reperfusion therapy, AMI still remains a high mortality rate, and the patients still face a mortality rate of up to 40% within 30 days[1]. Due to the lifestyle changes, the incidence of AMI is getting younger and younger[2]. Therefore, some new approaches were urgently needed to improve myocardial ischemic injury.

Ischemic cardiac injury triggers severe metabolic and ionic disorders, continuous ischemia results in the loss of many cardiomyocytes which cannot be replaced by the substantial loss of cardiomyocytes due to the adult mammalian heart's limited regenerative capacity[3,4]. Mitochondria are abundant in cardiomyocytes, providing energy for myocardial movement, regulating intracellular ion balance, and participating in apoptosis and necrosis[5]. Mitochondria are highly dynamic organelle in continuous fusion and division. The fusion and division of mitochondria in cardiomyocytes are used to meet the energy required for myocardial cell movement. Studies have shown that normal mitochondrial function plays an important role in hypoxia-induced myocardial injury. Abnormal fusion and fission of mitochondria can further cause mitochondrial dysfunction and lead to apoptosis[6]. Apoptosis is a common phenomenon after MI and mitochondria-mediated internal apoptotic pathway is the main cause of cardiomyocyte apoptosis[7,8]. Therefore, anti-apoptotic treatment of cardiomyocytes after MI can effectively prevent adverse remodeling and contractile dysfunction.

CD38 was discovered as a lymphocyte-specific antigen in lymphocytes. With the deepening of research, it was found that the protein existed in various cell types and organelles[9]. In mammals, CD38 is a major NAD^+ hydrolase

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that regulates intracellular NAD^+ levels. Previous research found that NAD^+ levels were obviously increased in CD38 knockout mice[10]. Besides, CD38 is also involved in the regulation of a number of physiological functions, such as the regulation of NAD^+ homeostasis and Ca^{2+} mobilization, as well as a number of diseases, including aging, obesity, nonalcoholic fatty liver disease, cardiac hypertrophy, diabetes and inflammation[11–14]. As an NAD^+-dependent deacetylase, Sirtuins have been linked to a wide range of cellular and physiological functions, including anti-oxidative stress, mitochondrial function regulation and cardiovascular disease[15]. Sirtuins family has seven members (Sirt1-Sirt7), among which Sirt3 is located in mitochondria and regulates mitochondrial lysine acetylation[16,17]. Mitochondrial dysfunction promotes the occurrence of cardiovascular diseases to a large extent, and Sirt3 has been found to be essential in a variety of cardiovascular diseases[18–20]. We previously found that CD38 played an important role in hypoxia-induced myocardial injury and CD38 deficiency protected against IRI. Meanwhile, CD38 deficiency protected against AngII-induced cardiac hypertrophy and inhibited cardiomyocyte senescence[21–23]. However, the effects of cardiomyocytes CD38 on acute myocardial infarction and the underlying mechanisms remain unknown.

In the present study, we examined the roles of CD38 in AMI and found cardiomyocyte-specific CD38 deficiency protected cardiac function after AMI. In addition, CD38 deficiency can reduce ischemia or hypoxia-induced apoptosis in vivo and in vitro. Furthermore, we demonstrated that cardiomyocyte-specific CD38 deficiency protected against AMI by reducing cardiomyocytes apoptosis via activating the Sirt3 signaling pathway.

Results

Cardiomyocyte-specific deletion of CD38 gene improves cardiac functions and reduces fibrosis in mice with acute myocardial infarction

To better understand the roles of CD38 in heart, the cardiomyocyte-specific CD38 knockout ($CD38^{CKO}$) mice were generated, in which the mice provided a useful tool for specifically examining the roles of CD38 in cardiomyocytes rather than other cell types such as macrophages and fibroblasts. The results showed that a high efficiency of CD38 deletion in heart tissue was obtained by western blot analysis (Fig. 1A) and the echocardiographic data showed that CD38 deficiency did not alter baseline heart function (Fig. 1B). To examine the roles of CD38 in AMI, 8-week-old-male $CD38^{flox/flox}$ and $CD38^{CKO}$ mice were used to construct AMI models. Morphological analysis showed that the ratio of heart weight to body weight (Fig. 1C) and heart weight to tibia length (Fig. 1D) in $CD38^{CKO}$ mice were lower than $CD38^{flox/flox}$ mice after 28 days of AMI, whereas there was no difference between the two genotypes in the sham-operation. The results indicated that cardiomyocyte-specific deletion of CD38 alleviated the cardiac hypertrophy in mice after AMI. In addition, the serum LDH level was significantly decreased in $CD38^{CKO}$ mice after AMI compared to the control group (Fig. 1E). Furthermore, there was no statistically significant difference in cardiac functions including LVIDs and LVIDd of $CD38^{CKO}$ mice compared with $CD38^{flox/flox}$ mice at day 3 in AMI (Fig. 2D and E). However, the LVIDs, LVIDd, EF and FS were significantly restored on the 7th day in AMI in $CD38^{CKO}$ mice compared with control mice (Fig. 2A—E). Fibrosis has a detrimental effect on ventricular remodeling in myocardial infarction. In our results, $CD38^{CKO}$ mice showed less fibrosis at the peri-infarct area in AMI compared with $CD38^{flox/flox}$ mice (Fig. 2F, H and I). Additionally, the anti-fibrosis gene IL-10 was significantly upregulated after CD38 deficiency (Fig. 2G and J). These results indicated that CD38 deficiency in cardiomyocytes significantly improved the cardiac functions and reduced fibrosis in acute myocardial infarction.

Cardiomyocyte CD38 deficiency attenuates cardiac apoptosis in AMI

Studies have shown that apoptosis is one of the crucial mechanisms of early myocardial injury in AMI, in which mitochondria-mediated apoptosis is the main cause of cardiomyocyte apoptosis. To elucidate the roles of CD38 deficiency in mitochondria-mediated apoptosis, the expression of pro-apoptosis protein Bax and anti-apoptosis protein Bcl2 were examined in the cytoplasm and mitochondria of cardiomyocytes in mice on day 7 after AMI. Our results found that CD38 deficiency obviously reversed AMI-induced decreases of the mitochondrial Bcl2 and Bax ratio, but there was no influence on the cytoplasmic Bcl2/Bax ratio. The results suggested that CD38 was involved in the translocation of Bax and Bcl2 in mitochondria, and CD38 deficiency reduced cardiomyocytic apoptosis by increasing the translocation of anti-apoptosis protein Bcl2 to mitochondria (Fig. 3A—C). In addition, on day 7 after AMI, the expression of proapoptotic molecule cleaved caspase-3 was significantly decreased in $CD38^{CKO}$ mice compared with $CD38^{flox/flox}$ mice, but there was no difference in pro-caspase-3 expression in heart tissues between the two groups (Fig. 3D—F). Moreover, our results also showed that CD38 deficiency in cardiomyocytes improved mitochondrial fusion abnormalities caused by AMI, seen as the increased expressions of Mfn1 and Mfn2 proteins in mitochondria (Fig. 3G—I), whereas there was no significant effect on mitochondrial fission (Fig. 3J and K). Taken together, these data suggested that CD38 deficiency in cardiomyocyte reduced AMI-induced cardiac apoptosis in vivo.

CD38 deficiency protects cardiomyocytes from hypoxia-induced cell death in vitro

To better understand the roles of CD38 in AMI in vitro, CD38 knockdown stable H9c2 cell line was treated with 1% O_2 to induce hypoxia injury. As shown in Fig. 4A and C by flow cytometric analysis, the apoptosis was increased after hypoxia, whereas CD38 knockdown significantly reduced hypoxia-induced cardiomyocyte apoptosis. Decreased mitochondrial membrane potential is one of the events of the early apoptosis. The J-aggregates of red fluorescence exists when the cells were cultured normally, when the mitochondrial membrane potential is decreased after hypoxia treatment, showing monomer of green fluorescence. Our results showed that the mitochondrial membrane potential was significantly decreased under hypoxia and existed in the form of green fluorescent monomer, whereas CD38 knockdown markedly inhibited mitochondrial membrane potential decline under hypoxia (Fig. 4B and D). In addition, the Bcl2/Bax ratio was significantly increased in

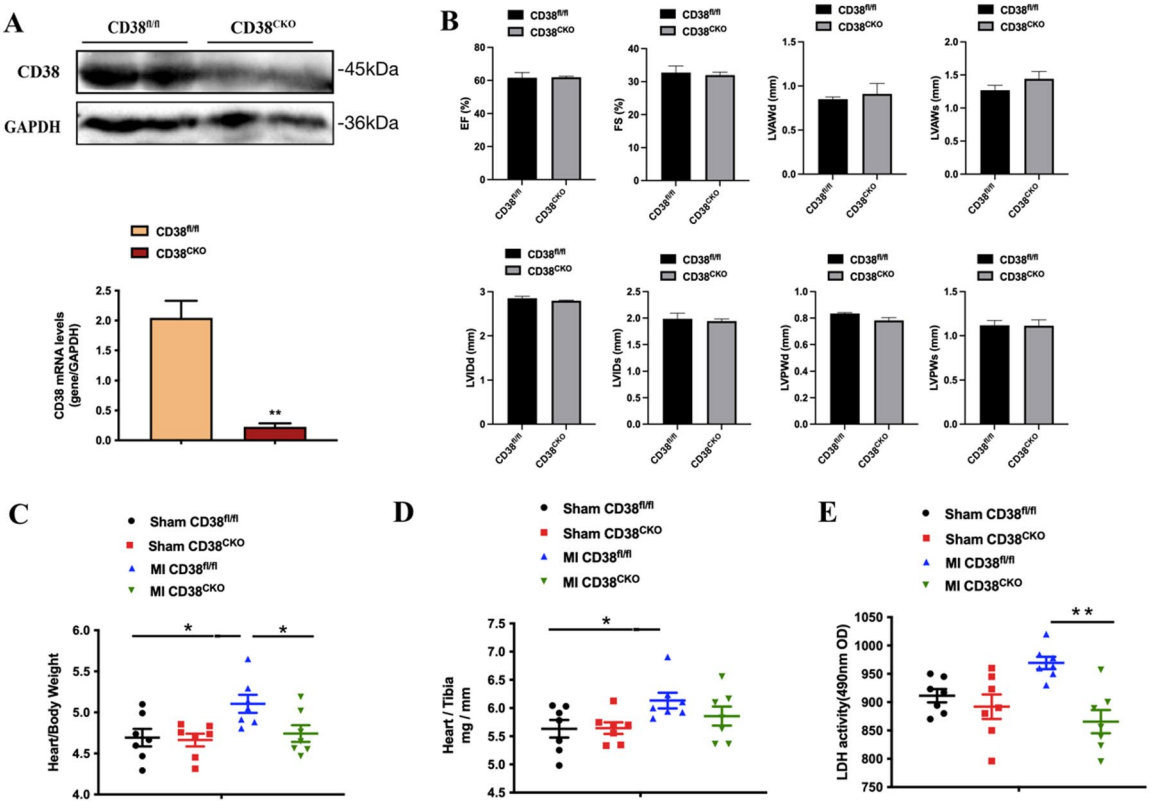

Fig. 1. Cardiomyocyte-specific CD38 deficiency mice do not alter baseline heart function. **A** The efficiency of CD38 knockout was confirmed by immunoblot analysis and RT-PCR. **B** Ventricular function measured by echocardiography in heart tissue from $CD38^{flox/flox}$ and $CD38^{CKO}$ mice heart tissues. **C** The heart weight (HW)/body weight (BW) was measured from $CD38^{flox/flox}$ and $CD38^{CKO}$ with sham or 28 days post-MI. **D** The heart weight (HW)/tibia length was measured from $CD38^{flox/flox}$ and $CD38^{CKO}$ with sham or 28 days post-MI. **E** The LDH activity in serum of $CD38^{flox/flox}$ and $CD38^{CKO}$ mice after 28 days post-MI. Data represent the means ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, $n = 7$ per group.

CD38 knockdown cardiomyocytes after hypoxia treatment compared with control groups (Fig. 4E—G). Taken together, these data suggested that CD38 knockdown in cardiomyocytes markedly alleviated hypoxia-induced apoptosis.

CD38 deficiency protects cardiomyocytes from hypoxia-induced cell death in vitro by mediating mitochondrial dysfunction

To further determine whether the effects of CD38 on cardiomyocytic apoptosis were related to mitochondria, the mitochondria were isolated from cardiomyocytes for further experiments. In our results, CD38 knockdown significantly reduced hypoxia-induced Bax translocation and increased the expression of anti-apoptotic molecule Bcl2 (Fig. 5A—C). Moreover, immunoblot analysis revealed that CD38 knockdown significantly reduced hypoxia-induced the increases of cleaved-caspase3 expression, whereas there was no influence on hypoxia-induced the increase of the pre-caspase-3 expression (Fig. 5D—F). Furthermore, CD38 knockdown remarkably increased the expressions of Mfn2, suggesting that CD38 knockdown improved mitochondrial fusion abnormalities caused by hypoxia (Fig. 5G and H). All these results further confirmed that CD38 knockdown in cardiomyocytes markedly alleviated hypoxia-induced cell death by mediating mitochondrial dysfunction.

Knockdown of CD38 reduces hypoxia-induced cardiomyocytic apoptosis through activating Sirt3 pathway

Mounting evidences indicate that Sirt3 located in mitochondria has protective effects on apoptosis. Our results showed that cardiomyocyte-specific CD38 deficiency increased Sirt3 protein levels on day 7 after AMI (Fig. 6A and B). Furthermore, CD38 knockdown increased Sirt3 protein expression under hypoxia in vitro (Fig. 6C and D). To further explore the protective mechanisms of CD38 on apoptosis after AMI, the effects of specific inhibitor of Sirt3 on hypoxia-induced cardiomyocytic apoptosis were examined in primary cardiomyocytes from $CD38^{flox/flox}$ mice and $CD38^{CKO}$ mice. According to Fig. 6E and 6F, 3-TYP (Sirt3 inhibitor) reduced hypoxia-

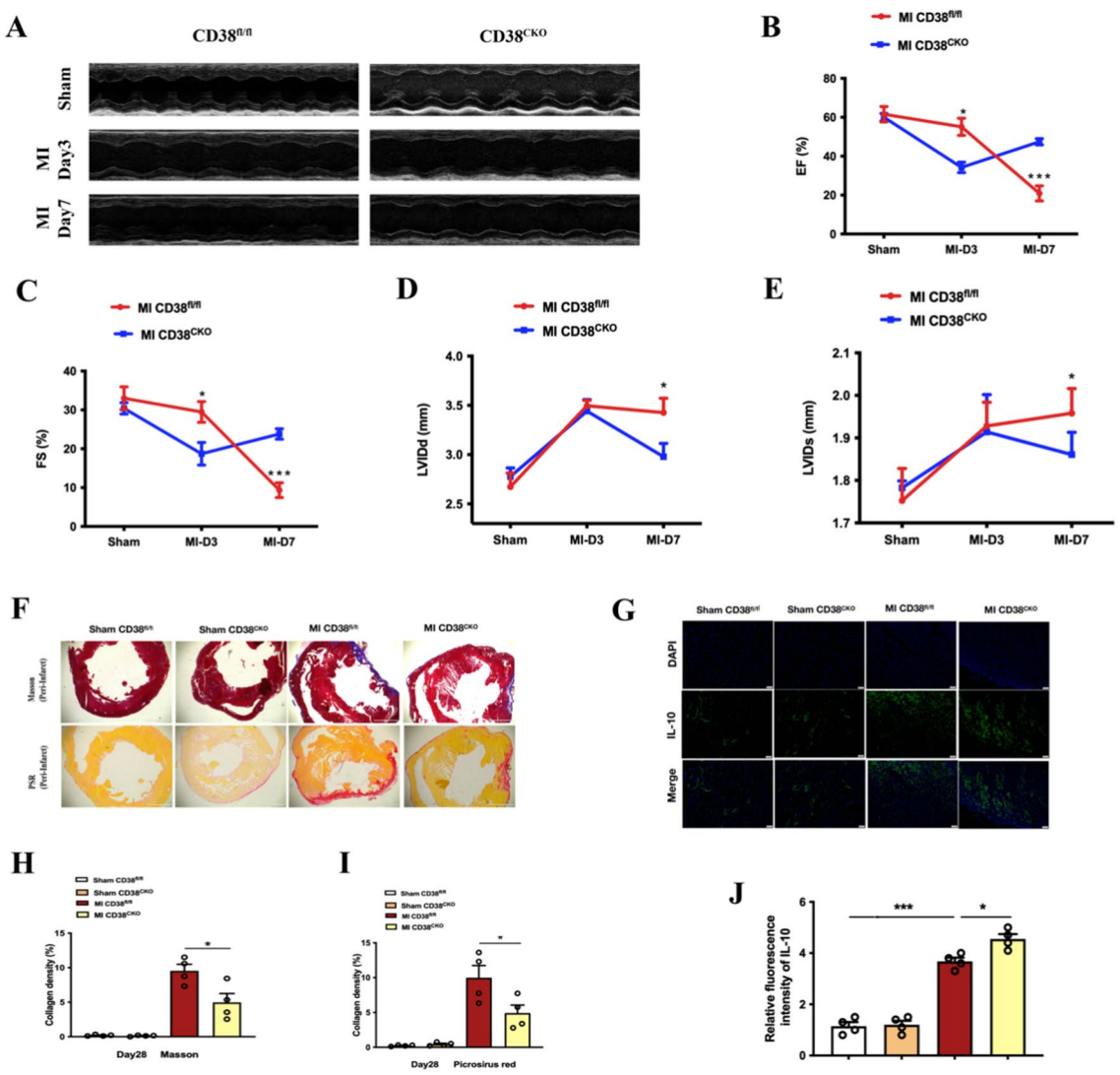

Fig. 2. Cardiomyocyte-specific CD38 deficiency protects cardiac function and reduces fibrosis in mice after acute myocardial infarction. **A** Representative echocardiograms obtained from $CD38^{flox/flox}$ and $CD38^{CKO}$ mice subjected to MI or sham surgery. **B**–**E** Echocardiographic measurements of ventricular functions in $CD38^{flox/flox}$ and $CD38^{CKO}$ mice hearts after sham operation or 3 and 7 days post-MI. **F** Masson trichrome (TC) and Picrosirius red (PSR) staining of peri-infarction zone were examined to detect collagen within the scar of $CD38^{flox/flox}$ and $CD38^{CKO}$ hearts at 28 days after MI. **H**, **I** Measurement collagen density in (**F**). IL-10 quantification was performed by immunofluorescence staining (**G**), and the immunofluorescence intensity was recorded and analyzed by ImageJ software (**J**). Data represent the means ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, $n = 4$–7 per group.

induced mitochondrial membrane potential in primary cardiomyocytes from $CD38^{CKO}$ mice, but there were no changes in hypoxia-induced mitochondrial membrane potential with 3-TYP in the cardiomyocytes from $CD38^{flox/flox}$ mice. More importantly, Sirt3 inhibitor significantly promoted hypoxia-induced cardiomyocyte apoptosis (Fig. 6G and H). All these results indicated that the role of CD38 in cardiomyocyte apoptosis after AMI was dependent on Sirt3 signaling pathway.

Discussion

With a better understanding of the pathogenesis of myocardial infarction and the application of new treatment strategies, the survival rate after AMI has significantly improved. However, the patients with AMI still face poor short-term survival[24]. Our previous studies showed that there was an improvement of cardiac function in CD38 deficiency mice under different models[21,22,25]. However, the roles of cardiomyocyte CD38 in AMI and its mechanism has not been explored. Here, we reported that $CD38^{CKO}$ significantly protected mice from AMI without influencing the baseline of the cardiac functions. Cardiomyocyte death in AMI triggers a strong inflammatory response, followed by a transition to a multistage repair response, in which damaged cardiomyocytes are mainly replaced with fibroblasts and myofibroblasts which forms fibrotic scars in the damaged area to adapt

Figure 3

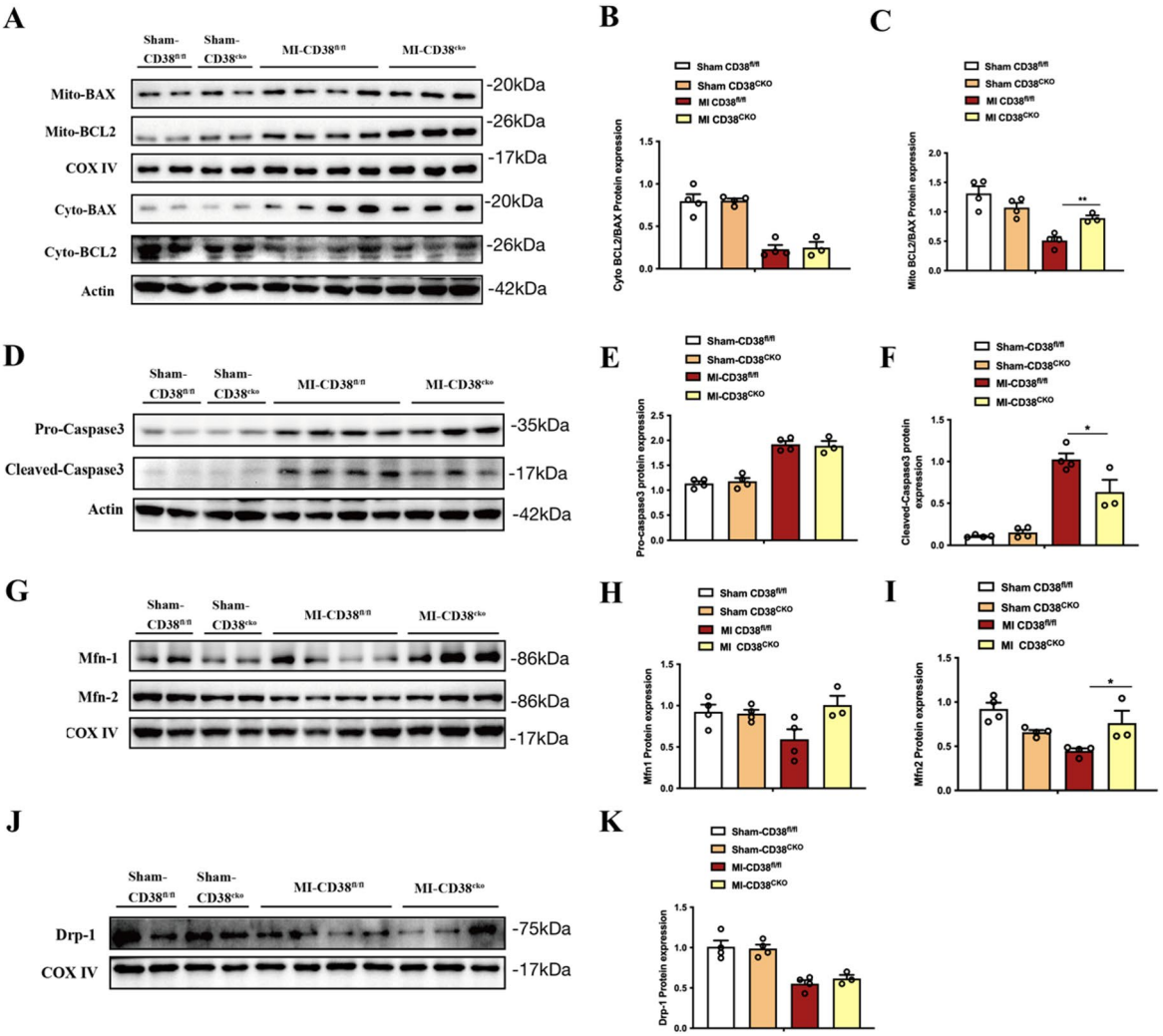

Fig. 3. Cardiomyocyte-specific CD38 deficiency attenuated cardiac apoptosis after MI. **A**–**C** Immunoblot analysis the expressions of Bax and Bcl2 proteins in mitochondria and cytoplasm in heart tissue of $CD38^{flox/flox}$ and $CD38^{CKO}$ mice at day 7 post-MI or post-sham operation. **D**–**F** The expressions of pro-Caspase3 and cleaved-Caspase3 proteins were determined by immunoblot analysis in heart tissue of $CD38^{flox/flox}$ and $CD38^{CKO}$ mice at day 7 post-MI or post-sham operation. **G**–**I** The expressions of Mfn2 and Mfn1 proteins in mitochondria were confirmed by immunoblot analysis in heart tissue of $CD38^{flox/flox}$ and $CD38^{CKO}$ mice at day 7 post-MI or post-sham operation. **J**, **K** Immunoblot analysis the expression of Drp1 protein in mitochondria in heart tissue $CD38^{flox/flox}$ and $CD38^{CKO}$ mice at day 7 post-MI or post-sham operation. Data represent the means ± SEM, $^{*}p < 0.05$ and $^{**}p < 0.01$, $n = 3$–4 per group.

to the myocardial tissue caused by ischemia[26,27]. Excessive reactive fibrosis at the peri-infarct area is an inevitable myocardial remodeling process after AMI and further develops into myocardial failure[28]. In the current study, we found that $CD38^{CKO}$ mice had markedly reduced fibrosis at the peri-infarct area compared with $CD38^{flox/flox}$ mice. These findings suggested that CD38 deficiency might improve cardiac function and reduce fibrosis at the peri-infarct area in AMI and protect against adverse remodeling in AMI.

In AMI, the reduction of blood flow in the myocardial area leads to the occurrence of infarction, and a large number of myocardial cells death[29]. Apoptosis of cardiomyocytes is the main pathologic mechanism leading to heart failure in AMI, and mitochondria-mediated apoptosis pathway is the main cause of inducing apoptosis of cardiomyocytes after myocardial infarction[5]. Herein, we observed that CD38 knockdown in H9c2 cells significantly reduced hypoxia-induced apoptosis compared to normal cells in vitro. In the meantime, the change of mitochondrial membrane potential is one of the early events of apoptosis. In our results, we found that CD38 knockdown in H9c2 cells markedly prevented hypoxia-induced mitochondrial membrane potential decline compared to normal cells in vitro. Furthermore, our study showed that CD38 deficiency exerted anti-apoptotic effects by reducing the expression of pro-apoptotic protein Bax and increasing the expression of anti-apoptotic protein Bcl2 in mitochondria in vitro and in vivo. And CD38 deficiency also inhibited the expression of cleaved caspase-3. After myocardial infarction, ischemia triggers the loss of a large number of cardiomyocytes, directly impairing the cardiac systolic function, while also releasing pro-inflammatory and pro-fibrotic factors

Figure 4

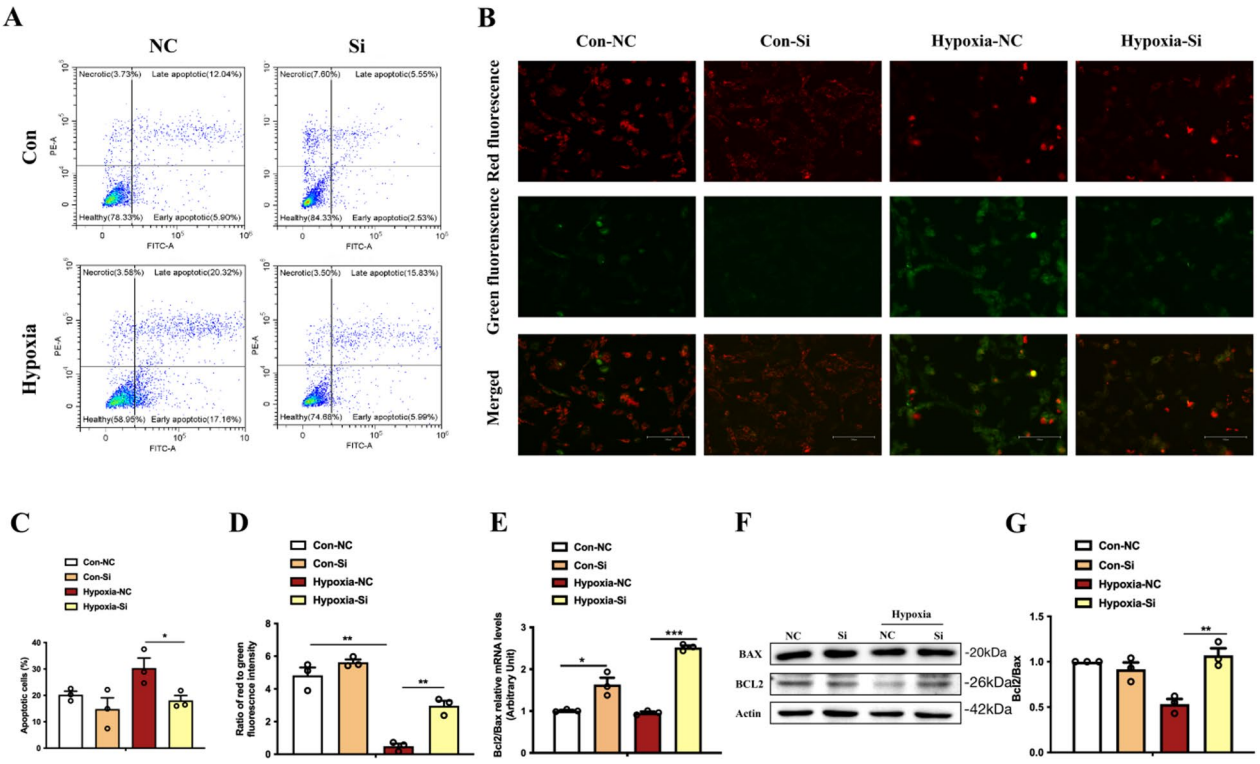

Fig. 4. CD38 deficiency protected cardiomyocytes from hypoxia-induced cell death in vitro. **A**, **C** Detection of apoptosis in H9c2 cells under hypoxia by flow cytometry. **B**, **D** Mitochondrial membrane potential of H9c2 cells treated with hypoxia (20X). **E** Bcl2/Bax mRNA expressions were quantified by RT-PCR in hypoxia-induced H9c2 cells. **F**, **G** Immunoblot analysis the expressions of Bcl2 and Bax proteins in H9c2 cells. Data represent the means ± SEM, $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, $n = 3$ per group.

that further promote fibrosis. In our results, we observed a reduction in cardiomyocyte apoptosis following CD38 deficiency, and consistent with this, we also observed a decrease in fibrosis in the peri-infarct area of infarcted myocardium. These results demonstrated that the anti-apoptotic effects of CD38 deficiency on AMI were mainly depended on protecting mitochondria-mediated apoptotic pathways.

Mitochondria play an important role in maintaining normal cell function. Abnormal mitochondrial fusion and division can affect mitochondrial function and lead to cell death[30,31]. Studies shown that myocardial mitochondria fission occurred during ischemia reperfusion injury, leading to mitochondrial dysfunction and eventually cell death[32]. Our in vivo study found that the expressions of myocardial mitochondrial fusion proteins Mfn1 and Mfn2 were significantly increased in $CD38^{CKO}$ mice on day 7 after AMI compared to $CD38^{flox/flox}$ mice, while the mitochondrial fission protein Drp1 showed no significant change. These findings demonstrated that CD38 deficiency protected against abnormal mitochondrial fusion after AMI.

Sirtuins are a class of deacetylases, including Sirt1-Sirt7, among which Sirt1 and Sirt3 have been widely studied. Sirt3, a soluble protein located on mitochondria, has been proven to play a protective role in a variety of cardiovascular diseases[18,33,34]. Studies shown that Sirt3 directly deacetylated cyclophilin protein D and delayed the opening of mPTP[35], suggesting that Sirt3 played a significant role in mitochondrial function regulation. In our study, we observed that Sirt3 expression was obviously upregulated in $CD38^{CKO}$ mice in AMI and CD38-deficient cardiomyocytes treated with hypoxia. More importantly, the Sirt3 inhibitor 3-TYP significantly aggravated hypoxic-induced apoptosis of CD38-deficient cardiomyocytes and decreased mitochondrial membrane potential, indicating that the anti-apoptotic effect of CD38 deficiency depended on the anti-apoptotic activity of NAD^+-mediated Sirt3 pathway.

Although this study revealed the potential role of CD38 deficiency in reducing cardiomyocyte apoptosis and fibrosis, there are still some limitations. Firstly, the experimental design was primarily based on animal models which might simulate the pathological processes of human myocardial infarction, but the species differences still exist. Future studies need to validate these findings with the clinical samples to further confirm the role of CD38 in human myocardial infarction. Second, the protective effect of CD38 deficiency on MI were examined at the specific time points after myocardial infarction in the present study, failing to comprehensively reflect the dynamic impact of CD38 deficiency on myocardial repair. Certainly, future research could analyze multiple time points to better understand the time-dependent effects of CD38 following myocardial infarction.

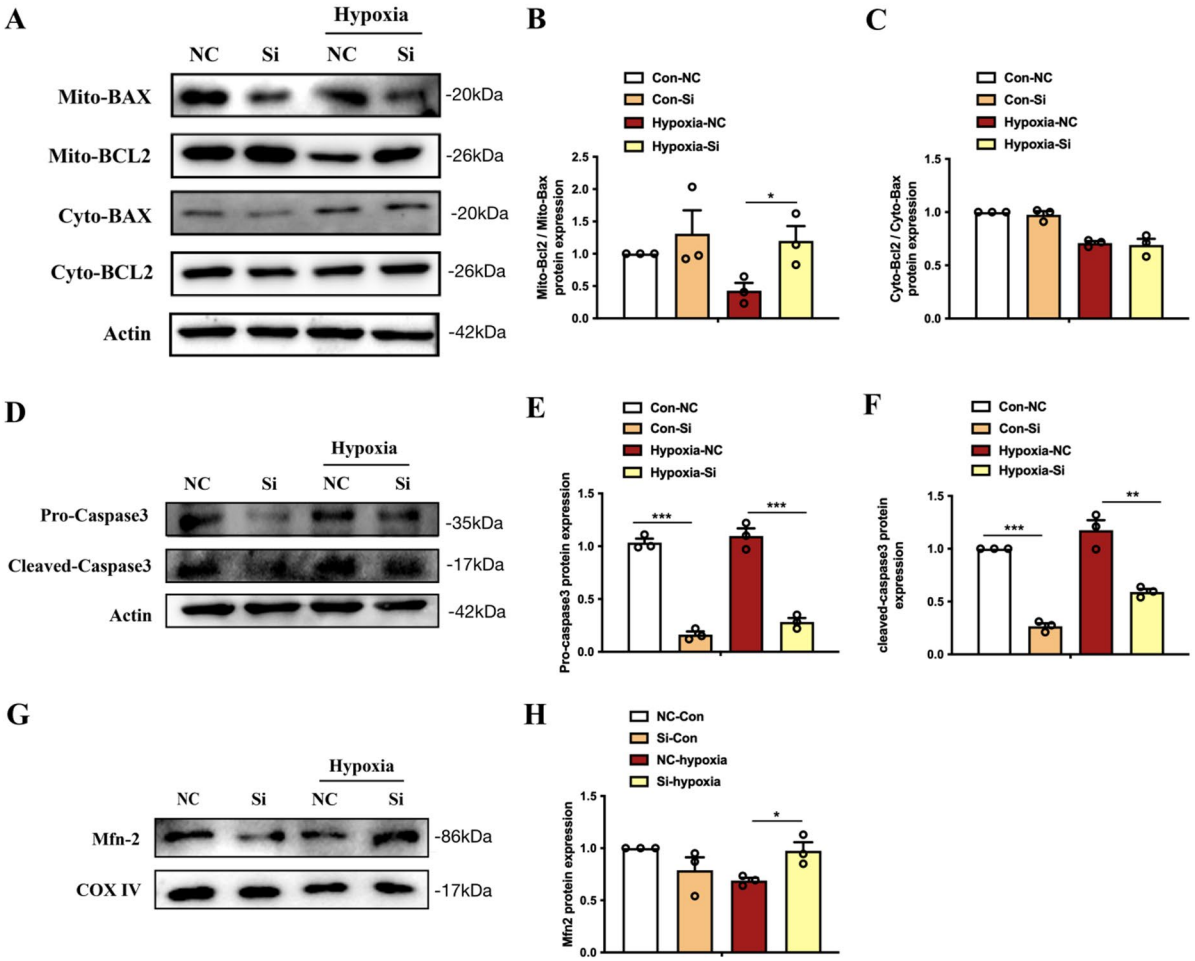

Fig. 5. CD38 deficiency protects cardiomyocytes from hypoxia-induced cell death in vitro by mediating mitochondrial dysfunction. **A**–**C** Immunoblot analysis the expressions of Bax and Bcl2 proteins in mitochondria and cytoplasm in H9c2 cells. **D**–**F** The expressions of pro-Caspase3 and cleaved-Caspase3 proteins were confirmed by immunoblot analysis in H9c2 cells. **G**, **H** Immunoblot analysis the expression of Mfn2 protein in H9c2 cells. Data represent the means ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, $n = 3$ per group.

In summary, our study demonstrated that cardiomyocyte specific CD38 protected the heart from AMI through reducing fibrosis at the peri-infarct and suppressing the apoptosis of cardiomyocytes. The mechanisms of CD38 deletion in cardiomyocytes protecting AMI might be related to activate Sirt3-mediated signaling pathway, in which hypoxia including AMI induces the apoptosis of cardiomyocytes by promoting the expression of mitochondrial Bax protein and inhibiting mitochondrial fusion, whereas cardiomyocyte CD38 deletion protects against AMI through elevating intracellular NAD^{+} levels which further activate the SIRT3 signaling pathway to produce their anti-apoptosis via increasing the Bcl2/Bax ratio and promoting mitochondrial fusion (Fig. 7). In addition, ischemia-reperfusion injury is also a primary clinical event for myocardial infarction. Our previous studies have demonstrated that CD38 deficiency protected heart from the ischemia-reperfusion injury through suppressing oxidative stress. The current study also revealed that CD38 deficiency alleviated MI through reducing the apoptosis of cardiomyocytes in the early stages of myocardial infarction. These results indicated that CD38 deficiency protected heart from MI and ischemia-reperfusion, suggesting that CD38 might play a critical role in MI or ischemia-reperfusion injury clinically. Our findings should contribute to a better understanding of the mechanisms underlying AMI and targeting CD38 may represent a promising therapeutic strategy for AMI prevention and treatment.

Materials and methods

Animal models

$CD38^{flox/flox}$ mice were crossed with MLC2v-Cre mice to generate cardiomyocyte-specific CD38 conditional knockout ($CD38^{CKO}$) mice. All mice were maintained on a C57BL/6 background, and this study used aged 6–8

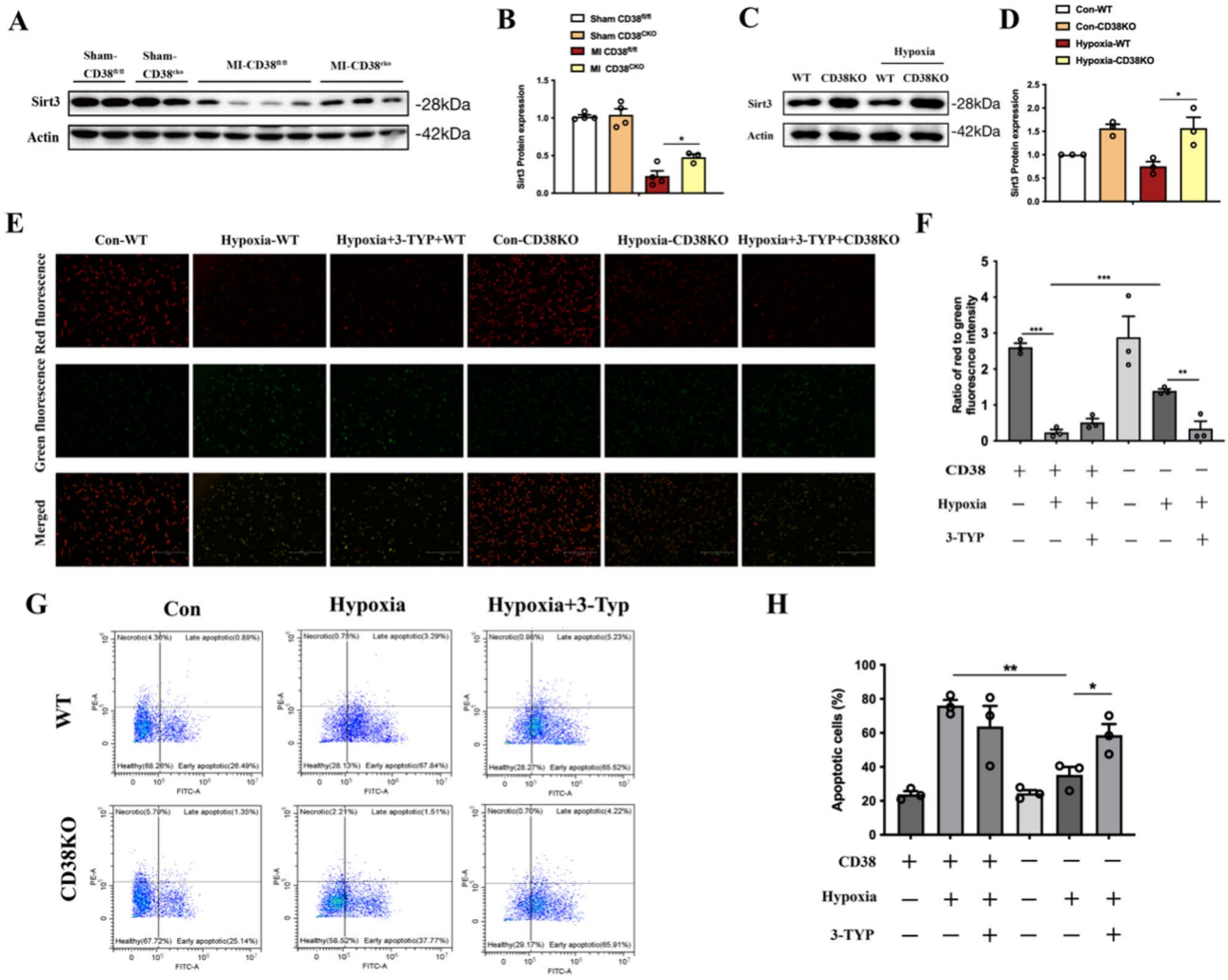

Fig. 6. CD38 deficiency reduced hypoxia-induced cardiomyocyte apoptosis through Sirt3 pathway. **A**, **B** Immunoblot analysis the expressions of Sirt3 protein in heart tissue of $CD38^{flox/flox}$ and $CD38^{CKO}$ mice at day 7 post-MI or post-sham operation. **C**, **D** Immunoblot analysis the expressions of Sirt3 protein in $CD38^{flox/flox}$ and $CD38^{CKO}$ mice primary cardiomyocytes. **E**, **F** Mitochondrial membrane potential of $CD38^{flox/flox}$ and $CD38^{CKO}$ mice primary cardiomyocytes treated with Sirt3 inhibitor 3-TYP (50μM). **G**, **H** Detection of apoptosis in $CD38^{flox/flox}$ and $CD38^{CKO}$ mice primary cardiomyocytes treated with 3-TYP (a Sirt3 inhibitor). Data represent the means ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, $n = 3$ per group.

weeks and only male mice were used in our experiments. $CD38^{flox/flox}$ and MLC2v-Cre mice were provided by Cyagen (Suzhou, China) and Dr. Hongliang Li (Wuhan University, China), respectively. The mice were anesthetized and fixed on the fixation plate with isoflurane (RWD Life Science), the fur was disinfected with alcohol, three or four ribs of the mice were separated with blunt instrument. The mice were gently opened the chest to expose the heart, and then ligated the left anterior descending coronary artery with 7 – 0 sterile line. Once the bottom of the heart was turned to white and then the chest was quickly sewed.

Cell culture and treatment

Primary cardiomyocytes and H9c2 (ATCC, CRL-1466™) cells (within 20 passages) were cultured in 37 °C high-glucose DMEM supplemented with 10% fetal bovine serum (Gibco) and 10% penicillin/streptomycin. Primary cardiomyocytes were isolated from 6 to 8 weeks $CD38^{flox/flox}$ and $CD38^{CKO}$ male mice, extracted as previously described in reference previously[36]. Cardiomyocytes were treated with 1% O_2 to induce hypoxia injury. After cell adherence, the cells were incubated in low-oxygen incubator for 12 h (air conditions are 5% CO_2, 1%O_2, 95% N_2). H9c2 cell lines with stable CD38 knockdown were created as previously described in reference[22]. At 70-80% confluence, the cells were incubated in low-oxygen incubator.

Echocardiography

The cardiac functions were measured by echocardiography (Vevo3100; Visual Sonics, Toronto, Ontario, Canada) on the 3rd and 7th days, respectively. Briefly, isoflurane anesthetizes the mice and laid on operating table warmed

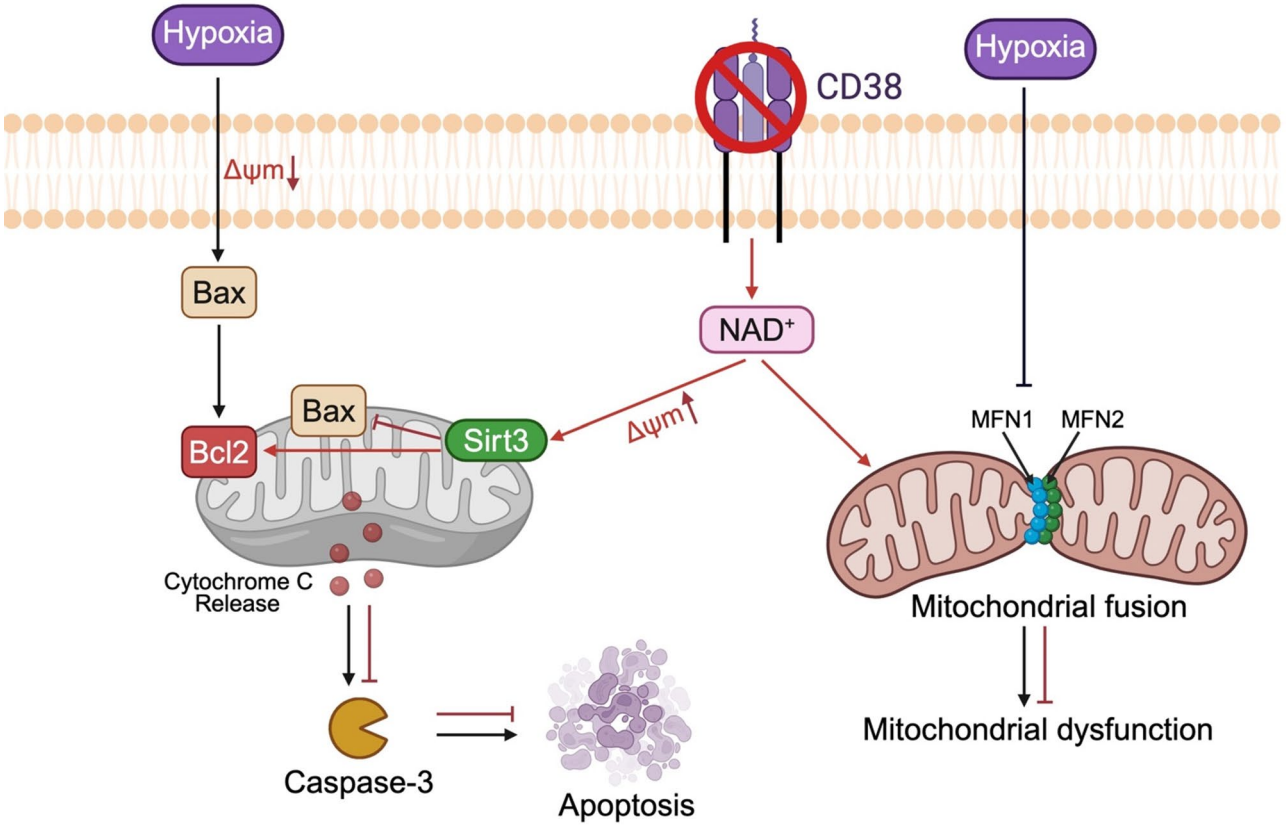

Fig. 7. Mechanisms of cardiomyocyte specific CD38 deletion protecting heart from acute myocardial infarction. Hypoxia including acute myocardial infarction (AMI) or myocardial ischemia induces the apoptosis of cardiomyocytes by promoting the expression of mitochondrial Bax protein and inhibiting mitochondrial fusion, whereas cardiomyocyte specific CD38 deletion protects against AMI through elevating intracellular NAD^+ levels which further activate the SIRT3 signaling pathway to produce their anti-apoptosis via increasing the Bcl2/Bax ratio and promoting mitochondrial fusion. Image was created by using the software from BioRender.com with the permission (agreement number: TC287E41WF; citation to use: https://BioRender.com/a9q2cc1).

to 37 °C, and the chest hair was removed using hair removing cream. Ventricular function, such as LV fractional shortening (FS) and LV ejection fraction (EF) were calculated according to instrument instructions.

Histology

Mice were euthanized with pentobarbital on day 28 after AMI, heart tissues were dissected, fixed in 4% paraformaldehyde overnight, rinsed with buffer followed by ethanol gradient dehydration, and samples embedded in paraffin. Section with a thickness of 5 μm were stained accordingly. Sirius red and Masson's trichrome staining showed fibrosis in the infarct area. Using ImageJ, color thresholds can be selected to further calculate the total tissue area and the positive area representing collagen fibers. Collagen density is then calculated as (positive area / total tissue area) × 100%.

Mitochondrial membrane potential assay

The MMP assay kit (Beyotime Biotechnology, China) was used to identify changes in mitochondrial membrane potential in primary cardiomyocytes and H9c2 cells. Briefly, primary cardiomyocytes and H9c2 cell line were cultured in 12-well plates. At a confluence of 70-80%, the cells were transferred to low-oxygen incubator (air conditions are 5% CO_2, 1%O_2, and 95% N_2). After hypoxia treatment, cells were stained with JC-1 dye for 20 min. The transition from red fluorescence to green fluorescence was detected by fluorescence microscope to estimate the decline of mitochondrial membrane potential. The experiment was performed at least three times.

LDH activity assay

LDH activities in serum of $CD38^{flox/flox}$ and $CD38^{CKO}$ mice after AMI were examined with LDH Assay Kit-WST (Dojindo, Japan). The supernatant was collected after centrifuging the mouse blood for 20 min at 1000 rpm. The supernatant was mixed with the Working Solution, incubated at room temperature for 20 min before adding the Stop Solution. The OD value was immediately detected with microplate reader at 490 nm.

Apoptosis detection
The apoptosis of H9c2 cells and primary cardiomyocytes after hypoxia was observed by apoptosis kit (Dojindo, Japan). Briefly, H9c2 cell line and primary cardiomyocytes were cultured in 6-well plates. At a confluence of 70-80%, the cells were transferred to low-oxygen incubator (air conditions are 5% CO_2, 1%O_2, and 95% N_2). The H9c2 cells and primary cardiomyocytes were stained with Annexin V-FITC and PI after hypoxia treatment, and flow cytometry was used to detect them.

Western blot analysis
Total tissue or cellular proteins were extracted by RIPA buffer containing 25 mmol/L HEPES, pH 7.4, 1% NP40, 137 mmol/L NaCl, 10% glycerol, 50 mmol/L NaF and 1mM protease inhibitor cocktail (Roche, Mannheim, Germany), the lysates were centrifuged at 13,000 rpm for 15 min. BCA Protein Assay Kit was used to detect the concentration of total protein extracted. Cells and tissues extracts were separated by 12% denaturing SDS-PAGE gels, and then transferred PVDF membranes, blocked with 5% skim milk powder at room temperature for 1 h, incubated with primary antibodies before being incubated with secondary antibodies. The primary antibodies against MFN1 (ABclonal, A9880), MFN2 (Proteintech, 12186-1-AP), Drp1 (Proteintch, 12957-1-AP), Bcl2 (CST, 3498 S), Bax (CST, 2772 S), Caspase-3 (CST, 9662 S) were used in this experiment. Secondary antibody was incubated at room temperature for 1 h and washed three times with TBST. At last, the luminescence was observed by ECL system. Mitochondrial proteins were extracted following the manufacturer's instruction (Beyotime, C2006).

RNA extraction and real-time RT-PCR
For each sample, total RNAs were extracted using the Trizol kit (Invitrogen). RNA concentration was measured using a spectrophotometer (Nanodrop 2000, Thermo Scientific), and RNA was converted to cDNA using the Takara reverse transcription kit. The ABI-Viia7 PCR machine was used for quantitative PCR. Q-PCR primer sequences were listed below: CD38(F- CTGCCAGGATAACTACCGACCT; R-CTTTCCCGACAGTGTTGCTTCT), Bax (F-AGGATGCGTCCACCAAGAAG; R-CCATATTGCTGTCCAGTTCATCTC), Bcl2(F- GTTGCAGTCACCGGATTCCT; R-CGGAGGTGGTGTGAATCCA), GAPDH (F-AGCCAAAAGGGTCATCATCT; R-GGGGCCATCCACAGTCTTCT). Sangon Biotech supplied all primer pairs (Shanghai, China).

Statistical analysis
Data were presented as mean ± SEM. The student's t test and two-way analysis of variance (ANOVA) were used to compare two or more groups. Statistical significances were showed as $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

Data availability
The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

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Author contributions

H-B.X., K-Y.D. and L-F.W led the project and contributed to the conception and design of the study, data analysis and interpretation, and manuscript revision. K.W performed most of the experiments and data analysis. Y-T.Z., Q-H. Z, Q.L., J-L.Z., Q.D., Y-F.X., X-H.G., M-X.J., Y-S.Q., and X-L.T. performed the animal models and cell experiments. K.W. and L-F.W. wrote the manuscript draft. All authors reviewed and approved the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

All the experimental procedures were approved by the Nanchang University Institutional Animal Research Committee. The animals used in this study were used according with the Jiangxi Provincial Standards for the Care of Laboratory Animals and in accordance with ARRIVE guidelines.

Additional information

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